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United States Patent [19]

Akhavan-Tafti

[11] **Patent Number:** **6,001,614**[45] **Date of Patent:** ***Dec. 14, 1999**[54] **METHODS OF SYNTHESIZING LABELED POLYNUCLEOTIDES BY LIGATION OF MULTIPLE OLIGOMERS**[75] **Inventor:** Hashem Akhavan-Tafti, Howell, Mich.[73] **Assignee:** Lumigen, Inc., Southfield, Mich.[*] **Notice:** This patent is subject to a terminal disclaimer.[21] **Appl. No.:** 09/241,353[22] **Filed:** Feb. 2, 1999

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[51] **Int. Cl.⁶** C12P 19/34; C12Q 1/68, C07H 21/04[52] **U.S. Cl.** 435/91.5; 435/6; 435/91.2; 435/91.52; 536/25.3; 536/25.32[58] **Field of Search** 536/25.3, 25.32; 435/6, 91.1, 91.2, 91.5, 91.52

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[57]

ABSTRACT

Methods of synthesizing polynucleotides are disclosed involving the simultaneous ligation of a set of oligomer 5'-phosphates onto a template-bound primer. The set of these oligomers can be preselected to contain oligomers which are complementary to the template strand or the oligomers can be supplied as a library and allowed to self select. The synthesis by ligation can proceed unidirectionally or bidirectionally from the primer and can be used to synthesize both strands simultaneously by the use of two primers. The ligation is preferably performed with a ligase enzyme. The methods of synthesis are useful in a variety of applications, including cloning, amplification, labeling, diagnostic assays, mutation analysis and screening, gene expression monitoring and sequence analysis.

23 Claims, 6 Drawing Sheets

